

THE INVENTION CLAIMED IS:

1. A medium of cell culture reagents, for the maintenance and growth of a pluripotent and/or germ line competent mammalian embryonic (ES) stem cell line, which medium is conditioned by a fibroblast cell clone that produces leukemia inhibitory factor.

2. The medium of claim 1, wherein the LIF producing fibroblast cell clone further comprises immortalized rabbit fibroblasts, and further comprises an animal serum or an animal serum replacement.

3. The medium of claim 1, wherein the cell culture reagents are selected from the group consisting of inorganic salts, amino acids, vitamins and sugars.

4. The medium of claim 2, wherein the serum is a fetal animal serum.

5. The medium of claim 2, wherein the serum is a newborn animal serum.

6. The medium of claim 1, comprising reagents selected from the group consisting of Phosphate Buffered Saline (PBS); Dulbecco's Modified Eagle Media (DMEM); Iscove's Modified Media; Dulbecco's Media; McCoy's 5A Media; Minimum Essential Media Eagle (MEM); RPMI Media 1640; Medium 199; MCDB Medium; RPMI; Glasgow Minimum Essential Media (GMEM); DMEM/F-12 Media; Hams F-10 Nutrient Mixture; Lebovitz's L-15 Media; CMRL Media; BGJb Medium; Basal Medium Eagle (BME); Brinster's BMOC-3 Medium; Williams Media E; and McCoy's Media.

7. The medium of claim 4, wherein the fetal animal serum is fetal bovine serum (FBS).

8. The medium of claim 7, wherein the fetal bovine serum is treated by a treatment selected from the group of dialysis, gamma irradiation or heat inactivation.

9. The medium of claim 1, further comprising a reducing agent.

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10. The medium of claim 9, wherein the reducing agent is selected from the group consisting of 2-mercaptoethanol and microhydrin.

11. The medium of claim 1, further comprising an antibiotic.

12. The medium of claim 11, wherein the antibiotic is selected from the group consisting of penicillin, streptomycin and gentamycin.

13. The medium of claim 1, further comprising L-glutamine.

14. The medium of claim 1, further comprising EGTA.

15. The medium of claim 1, further comprising albumin.

16. The medium of claim 4, wherein the serum is derived from an animal selected from the group consisting of sheep, pigs, chickens and horses.

17. The composition of claim 16, wherein the immortalized fibroblasts have been transfected, transformed or infected by a vector overexpressing a LIF gene.

18. The composition of claim 17, wherein the LIF gene is a rabbit LIF gene.

19. The composition of claim 18, wherein the fibroblast cell line used for conditioning is the Rab9 #19 cell line, which has been deposited with the Belgian Coordinated Collection of Microorganisms, under accession number LMBP 5479 CB.

20. A process of culturing mammalian ES stem cells to obtain pluripotent and/or germ line competent ES cells, wherein the culturing of the mammalian ES stem cells is at least partially performed in a composition as claimed in claim 1.

21. The process of claim 20, comprising the steps of:

a) culturing cells of blastocyst stage embryos;

- b) culturing isolated inner mass cells; and
c) passaging the inner mass cells periodically in a composition as claimed in claim 1.

22. The process of claim 21, wherein the inner mass cells are periodically passaged for at least eight times.

23. The process of claim 20, further comprising the step of producing transgenic animals.

24. Embryonic stem (ES) cell line with germ line transmission capability.

25. The cell line according to claim 20, which has germ line transmission capability after 11 or more passages.

26. The cell line of claim 24, obtainable by the process of claim 20.

27. The cell line of claim 24, wherein the cell line is a murine cell line.

28. The cell line of claim 27, wherein the cell line has been derived from cells or tissues with 129/SvEv; C57BL/6N; C57BL/6J-HPRT; BALB/c; CBA/CaO1a; 129/SvJ; DBA/2n; DBA/1 Ola; C3H/HeN; C57B1; 6Jol1; FVB; or Swiss Webster genetic backgrounds.

29. The cell line of claim 28, which has a germ line transmission capability after 11 or more passages.

30. The cell line of claim 29, wherein the cell line is cultured in a composition as claimed in claim 1 supplemented with cytokines and growth factors.

31. Embryonic stem (ES) cell line of claim 24, characterized by three-dimensional colony formation, positive staining for alkaline phosphatase; and negative staining for cytokeratin 18 and vimentin after more than 10 passages.

32. Embryonic stem (ES) cell line of claim 24, for use in the generation of chimeric or ES cell derived animals.

33. Embryonic stem (ES) cell line of claim 24, alteration by homologous or non-homologous recombination.

34. Embryonic stem (ES) cell line of claim 24, for use in the generation of animals with gene alteration via germ line transmission.

35. The method of using the ES cell line of claim 24, for generation of chimeric animals.

36. The method of using the ES cell line of claim 35, for the generation of chimeric animals following blastocyst injection into recipient blastocysts or embryo aggregation or nuclear transfer.

37. The method of differentiating the ES cell line of claim 24, for the study or isolation of (novel) genes.

38. The method of using the ES cell line of claim 24, for the expression or overexpression of genes.

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